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The role of clusterin (CLU) in extracellular protein folding quality control

Amy Ruth Wyatt
University of Wollongong

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By

Amy Ruth Wyatt

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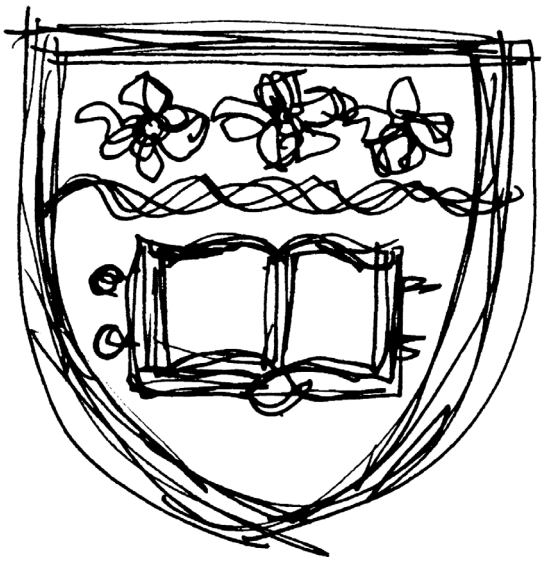

School of Biological Sciences
University of Wollongong
Wollongong, Australia

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DECLARATION OF AUTHENTICITY

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfilment of the Degree of Doctor of Philosophy. It does not include any material published by another person except where due reference is made in the text. The experimental work described in this thesis is original and has not been submitted for a degree to any other university.

Amy Ruth Wyatt

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ABSTRACT

Processes to attain and maintain the correct three-dimensional shape, known as the native conformation, of proteins are vital. However, certain conditions including thermal and oxidative stress may cause proteins to partially unfold and aggregate. Intracellular and/or extracellular protein aggregates have been identified in a large number of diseases, including Alzheimer's disease, arthritis and type II diabetes. While intracellular quality control for the folding state of proteins is well characterized, corresponding mechanisms for extracellular protein folding quality control have yet to be described.

Clusterin (CLU) is an abundant extracellular chaperone that can stabilize proteins and prevent their precipitation during exposure to elevated temperatures or oxidative stress *in vitro*. The work described here demonstrates that CLU stabilizes stressed client proteins by forming soluble, high molecular weight (HMW) complexes with them. The maximum loading of CLU appears to be at a mass ratio of CLU:stressed client protein of approximately 1:2 (irrespective of the identity of the client protein or the temperature used to induce heat stress). It was demonstrated that various human plasma proteins show increased association with CLU after plasma is subjected to mild shear stress or oxidative stress at 37°C - the most abundant of these was fibrinogen (FGN) which co-purified with CLU from stressed plasma. *In vitro*, using purified proteins, heat stress of 45°C for 12 h was required to induce FGN precipitation and the formation of HMW CLU-FGN complexes. Size exclusion chromatography (SEC) of the stressed plasma suggested that a portion of the complexes formed in plasma between CLU and FGN may be similar in mass to those formed *in vitro*.

Using surface plasmon resonance, although CLU was found to bind to megalin, only minimal (or no) binding of HMW complexes formed between CLU and glutathione-S-transferase, citrate synthase or lysozyme was detected. Similarly, negligible binding of these complexes to low density lipoprotein receptor superfamily members expressed on the surface of the rat yolk sac cell line BN was detected. However, the complexes were shown to preferentially bind to the surface of BN cells, peripheral human monocytes and rat hepatocytes (more so than uncomplexed CLU or client proteins). In all cases, this binding was inhibited by fucoidin (a scavenger receptor inhibitor). Confocal microscopy suggested that binding of HMW CLU-stressed protein complexes to the

surface of BN cells or rat hepatocytes was followed by their internalization into lysosomes. Furthermore, Western blotting showed that hepatocytes were able to degrade the HMW CLU-stressed protein complexes and that the degradation was almost completely abolished by inhibiting lysosomal proteases with chloroquine. The results of *in vivo* biodistribution studies in Sprague Dawley rats were highly consistent for several different HMW CLU-stressed protein complexes. Intravenous ^{123}I-labelled HMW CLU-stressed protein complexes were cleared more efficiently from circulation compared to free CLU and the uncomplexed client proteins. The liver and to a lesser degree the spleen appeared to be the key organs responsible for the uptake of complexes and this uptake was inhibited by pre-injection of the animals with fucoidin.

The findings of this study suggest an important role for CLU in global quality control of extracellular protein folding. It appears likely that stressed (partially unfolded) extracellular proteins are stabilized and held in solution by CLU and that CLU-stressed protein complexes are subsequently taken up by fucoidin-inhibitable cell surface receptors for subsequent degradation within lysosomes. The precise physical characteristic or binding site that targets CLU-stressed protein complexes for receptor-mediated uptake remains to be identified and further work is needed to determine the particular receptor(s) involved. The findings of this study support a model in which complexation with CLU is an important first step in the targeted disposal of stressed proteins via scavenger-like endocytic receptors.

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ABBREVIATIONS

ºC	degrees celsius
41D	monoclonal anti-clusterin antibody
Å	angstrom
α_2M	α_2-macroglobulin
A280	absorbance at 280 nm
A360	absorbance at 360 nm
A490	absorbance at 490 nm
ACID GLY	α_1 acid glycoprotein
AcLDL	acetylated low density lipoprotein
AFU	arbitrary fluorescence units
AGE	advanced glycation end product
AIF	apoptosis inducing factor
ALEXA488	Alexa fluor® 488
ALEXA633	Alexa fluor® 633
AMD	age-related macular degeneration
ApoAI	apolipoprotein AI
ApoE	apolipoprotein E
ApoER2	apolipoprotein E receptor 2
ASF	asialofetuin
ATP	adenosine triphosphate
Az	sodium azide
Aβ	amyloid-beta
BD	Becton, Dickinson and Company
bisANS	4,4'-Bis(1-anilino-8-naphthalene sulfonate)
BSA	bovine serum albumin
CD	circular dichroism
CLU	clusterin
COL	collagenase IV
CPK	creatine phosphokinase
CS	citrate synthase
CSF	cerebral spinal fluid
Da	daltons
DCM	dichloromethane
DLS	dynamic light scattering
DMEM:F-12	Dulbecco's modified Eagles medium: Hams F-12
DMSO	deoxymethylsulfoxide
DNA	deoxyribonucleic acid
dSR-CI	drosophila scavenger receptor class C type I
DTT	dithiothreitol

E. coli	*Escherichia coli*
ECL	enhanced chemiluminescence
EDTA	ethylene diamine tetraacetic acid
EGF	epidermal growth factor
EGTA	ethylene glycol tetraacetic acid
ELISA	enzyme linked immunosorbent assay
Em	emission
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum-associated protein degradation
Ex	excitation
F	F statistic
FCS	fetal calf serum
FEEL-1	fasciclin EFG-like, laminin-type EFG-like, and link domain-containing scavenger receptor type 1
FEEL-2	fasciclin EFG-like, laminin-type EFG-like, and link domain-containing scavenger receptor type 2
FGN	fibrinogen
FITC	fluorescein isothiocyanate
FPLC	fast protein liquid chromatography
g	relative centrifugal force (9.8 $m.s^{-2}$)
G7	monoclonal anti-CLU antibody
GST	glutathione-S-transferase
GST-RAP	glutathione-S-trasferase-receptor-associated protein (fusion protein)
h	hour(s)
HBB	Hank's binding buffer
HDC	heat denatured casein
HDC/PBS	1% heat denatured casein and 0.01% thimerosal in phosphate buffered saline.
HDL	high density lipoprotein
HMW	high molecular weight
Hp	haptoglobin
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
HSA	human serum albumin
Hsc	heat-shock cognate protein
HSD	honestly significant difference
Hsp	heat-shock protein
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin

IPTG	isopropyl-β-D-thiogalactopyranoside
K_d	dissociation constant
kDa	kilo daltons
Lamp 2a	lysosome-associated membrane protein type 2a
LB	Luria-Bertani
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LDLR	low density lipoprotein receptor
LOX-1	lectin-like oxidized low density lipoprotein receptor
LRP	low density lipoprotein receptor-related protein
ly-hsc73	lysosomal hsc73
LYS	lysozyme (hen egg)
MAC	membrane attack complex
MARCO	macrophage receptor with collagenous structure
mBSA	maleylated bovine serum albumin
mg	milligrams
min	minutes
mL	millilitres
mm	millimetres
mM	millimolar
Mr	marker
MW	molecular weight
m/z	mass to charge
µg	micrograms
µL	microlitres
µm	micrometres
µM	micromolar
n	number
NAMIDD	non-amyloidotic monoclonal IgG deposition disease
ng	nanograms
nm	nanometres
nM	nanomolar
NRD	no reported data
OPD	*o*-phenylenediamine dihydrochloride
OVA	ovalbumin
oxLDL	oxidized low density lipoprotein
PARP	poly ADP ribose polymerase
p	statistical significance
p.i.	post-injection
PBS	phosphate buffered saline
PDDs	protein deposition diseases

PEG	polyethylene glycol
pH	power of hydrogen
PI	propidium iodide
RAGE	receptor for advanced glycation end products
RAP	receptor-associated protein
ROS	reactive oxygen species
rpm	revolutions per min
s	seconds
SA	streptavidin
SAP	serum amyloid P component
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
sHsps	small heat-shock proteins
SOD	superoxide dismutase
SPECT	single photon emission computed tomography
SR-AI	scavenger receptor class A type I
SR-AII	scavenger receptor class A type II
SR-AIII	scavenger receptor class A type III
SR-BI	scavenger receptor class B type I
SRCR	scavenger receptor cysteine-rich domain
SREC-1	scavenger receptor expressed by endothelial cells
SR-PSOX	scavenger receptor for phosphatidylserine and oxidized low density lipoprotein
TAE	TRIS acetate EDTA
TEM	transmission electron microscopy
TMED	*N,N,N',N'*-tetramethylethylenediamine
TRANS	transferrin
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
tRNA	transfer riboneucleic acid (not defined in text)
TRYP	α_1 antitrypsin
UPR	unfolded protein response
UV	ultraviolet
V	volts
v/v	volume per volume
VLDL	very low density lipoprotein
VLDLR	low density lipoprotein receptor
V_o	void volume, exclusion limit
w/v	weight per volume
Z	Z score

PUBLICATIONS AND CONFERENCE PRESENTATIONS

Publications

Yerbury, J. J., Stewart, E. M., Wyatt, A. R. and M. R. Wilson (2005) Quality control of protein folding in extracellular space. *EMBO Reports* 6: 1131-1136

Wyatt, A. R., Yerbury, J. J., Poon, S. and M. R. Wilson (2009) Therapeutic targets in extracellular protein deposition diseases. *Current Medicinal Chemistry* 16(22): 2855-2866

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Conference Presentations

2006 International Biotechnology and Medical Science Student Forum, Beijing, China.
Oral presentation titled: The role of clusterin in extracellular protein quality control.

2007 World Conference of Stress, Budapest, Hungary.
Poster presentation titled: Its time to take out the trash! – Chaperone-dependent disposal of unfolded proteins via LDL superfamily receptors.

2007 International Society of Neurochemistry, Protein Misfolding and Neurodegenerative Disease Meeting, Dunk Island, Australia.
Oral presentation titled: The role of clusterin in extracellular protein quality control.

2008 5th Clusterin/ApoJ Workshop, Spetes, Greece
Oral presentation titled: The role of clusterin in extracellular protein quality control.

2008 AINSE 50th Anniversary Seminar Series, Lucas Heights, Australia
Oral presentation titled: The role of clusterin in extracellular protein quality control.